Leptospira Medium Base, Korthof, Modified

M457

Leptospira Medium is used for isolation, cultivation and maintenance of *Leptospira* species.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	0.800
Sodium chloride	1.400
Sodium bicarbonate	0.020
Potassium chloride	0.040
Calcium chloride	0.040
Monopotassium hydrogen phosphate	0.240
Disodium hydrogen phosphate	0.880
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

1)Preparation of Base:

Suspend 3.42 grams of M457 in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Distribute in 100 ml amounts in flasks. Sterilize by autoclaving at 115°C for 15 minutes. Cool to 55°C.

2) Preparation of Haemoglobin Solution:

To the rabbit blood clot, after removing serum, add equal volume of distilled water. Freeze and thaw repeatedly to haemolyse the corpuscles. Sterilize by Seitz or millipore filtration.

3) Complete Medium:

To 100 ml sterile base, add sterile 8 ml inactivated blood serum and 0.8 ml sterile haemoglobin solution. Mix thoroughly. Distribute if desired in 2-3 ml amount in sterile screw capped Bijou bottles/tubes. Test for sterility by incubating at 37°C.

Principle And Interpretation

Leptospirosis is an acute febrile disease caused by members of the genus *Leptospira* (1,2). Direct culture of blood is the most reliable way to detect *Leptospira* during the first week of illness. After the first week of illness and for several months thereafter, leptospires may be isolated by direct culture of undiluted urine specimens. By autopsy, leptospires may be isolated from kidney and liver tissues as well as from blood and urine. Leptospira Medium Base, Korthof, Modified is formulated as described by Korthof (3, 4) for cultivation and maintenance of *Leptospira* species.

Peptic digest of animal tissue provide amino acids and other nitrogenous substances to support bacterial growth. Haemoglobin solution and inactivated blood serum provide additional sources of nutrients to the Leptospires. The salts supply essential nutrients for the growth of the organisms. Phosphates form buffering system while sodium chloride maintains osmotic equilibrium and also provides essential ions.

All cultures are incubated at room temperature in the dark for up to 6 weeks. The organisms grow below the surface. Material collected from a few centimeters below the surface of broth cultures should be examined weekly for the presence of growth using a direct wet preparation under dark field illumination. Letpospires will exhibit corkscrew like motility (1).

Examine the tubes for growth every 5-7 days. Growth occurs as a ringed area (disc) 1-3 cm below the surface of the medium. The absence of a ringed area of growth doesnt necessarily mean leptospires are not present. Remove a small amount of growth from the disc area and examine microscopically (gram stain is not satisfactory). Microcolonies can be fixed with methanol and stained with Giemsa's stain to show rod forms (3).

Quality Control

Appearance
Off-white to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Yellowish brown coloured, clear to slightly opalescent solution after addition of serum and haemogloin

Reaction
Reaction of 0.342% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH
7.00-7.40

Cultural Response
M457: Cultural characteristics observed with added inactivated blood serum and sterile haemoglobin solution, after an incubation at 30°C for upto 2-7days.

Organism	Growth
Leptospira interrogans sero.grippotyhosa	luxuriant
Leptospira interrogans sero. Australis	luxuriant
Leptospira interrogans sero. Canicola	luxuriant

Storage and Shelf Life
Store below 30°C in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

Reference
1.Forbes B. A., Sahm A. S., and Weissfeld D. F., Bailey & Scotts Diagnostic Microbiology, 10th Ed., 1998, Mosby, Inc., St. Louis, Mo.
2.Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
3.Korthof G., 1932, Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. I. Orig., 125:429.
4.Rechcigl M. Jr. (Ed.), 1978, Handbook Series in Nutrition and Food, Vol. III, CRC Press.

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